HYPOGONADISM IN MALE CANCER PATIENTS:
A CROSS-SECTIONAL STUDY

by

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DEDICATION

To my parents- Dr. Dargaiah Konda and Dr. Ahalya Konda
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by

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HYPOGONADISM IN MALE CANCER PATIENTS:
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Bhavana Konda MD, MPH
The University of Texas
School of Public Health, 2010
Thesis Chair: Jose M Garcia, MD

ABSTRACT

Introduction
Cancer is the second most common cause of death in the USA (2). Studies have shown a coexistence of cancer and hypogonadism (8,29,11). The majority of patients with cancer develop cachexia, which cannot be solely explained by anorexia seen in these patients. Testosterone is a male sex hormone which is known to increase muscle mass and strength, maintain cancellous bone mass, and increase cortical bone mass, in addition to improving libido, sexual desire, and fantasy (12). If a high prevalence of hypogonadism is detected in male cancer patients, and a significant difference exists in testosterone levels in cancer patients with cachexia versus those without cachexia, testosterone may be administered in future randomized trials to help alleviate cachexia.

Study group and design
The study group consisted of male cancer patients and non-cancer controls aged between 40 and 70 years. The primary study design is cross-sectional with a sample size of 135. The
present data analysis is done on a subset convenience sample of 72 patients recruited between November 2006 and January 2010.

Methods

Patients aged 40-70 years with or without a diagnosis of cancer were recruited into the study. All patients with a BMI over 35, significant edema, non-melanomatous skin cancer, current alcohol or illicit drug abuse, concomitant usage of medications interfering with gonadal axis, and anabolic agents, patients on tube feeds or parenteral nutrition within 3 months prior to enrollment were excluded from the study. The study was approved by the Institutional Review Board of Baylor College of Medicine and is being conducted at the Michael E. DeBakey Veterans Affairs Medical Center at Houston. My thesis is a pilot data analysis that employs a smaller subset convenience sample of 72 patients determined by using the data available for the 72 patients (of the intended sample of 135 patients) recruited between November 2006 and January 2010. The primary aim of this analysis is to compare the proportion of patients with hypogonadism in the male cancer and non-cancer control groups, and to evaluate if a significant difference exists with respect to testosterone levels in male cancer patients with cachexia versus those without cachexia. The procedures of the study relevant to the current data analysis include blood collection to measure levels of testosterone and measurement of body weight to categorize cancer patients into cancer cachexia and cancer non-cachexia sub-groups.

Results

After logarithmic transformation of data of cancer and control groups, the unpaired t test with unequal variances was done. The proportion of patients with hypogonadism in the male cancer and non-cancer control groups was 47.5% and 22.7% with a Pearson chi2 statistic of 1.6036 and a p value of 0.205. Comparing the mean calculated bioavailable testosterone in
male cancer patients and non-cancer controls resulted in a t statistic of 21.83 and a p value less than 0.001. When the cancer group alone was taken, the mean free testosterone, calculated bioavailable testosterone and total testosterone levels in the cancer non-cachexia sub-group were 3.93, 5.09, 103.51 respectively and in the cancer cachexia sub-group were 3.58, 4.17, 84.08 respectively. The unpaired t test with equal variances showed that the two sub-groups had p values of 0.2015, 0.1842, and 0.4894 with respect to calculated bioavailable testosterone, free testosterone, and total testosterone respectively.

Conclusions

The small sample size of this exploratory study, resulting in a small power, does not allow us to draw definitive conclusions. For the given sub-sample, the proportion of patients with hypogonadism in the cancer group was not significantly different from that of patients with hypogonadism in the control group. Inferences on prevalence of hypogonadism in male cancer patients could not be made in this paper as the sub-sample is small and therefore not representative of the general population. However, there was a statistically significant difference in calculated bioavailable testosterone levels in male cancer patients versus non-cancer controls. Analysis of cachectic and non-cachectic patients within the male cancer group showed no significant difference in testosterone levels (total, free, and calculated bioavailable testosterone) between both sub-groups. However, to re-iterate, this study is exploratory and the results may change once the complete dataset is obtained and analyzed. It however serves as a good template to guide further research and analysis.
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BACKGROUND

Literature Review

Cancer- Epidemiology

Cancer is the second leading cause of death in the USA (2). More than 1.5 million Americans are diagnosed with cancer every year (5). About 23% of all causes of deaths in the USA are secondary to cancer (15). The estimated new cancer cases for 2009- based on the North American Association of Central Cancer registries from 1995 to 2005- is $7.66 \times 10^5$ in males and $7.13 \times 10^5$ in females (2). Cancer of the lung and bronchus is by far the most common cause of deaths due to cancer in both men and women accounting for 30% and 20% of all cancer deaths in the two sexes respectively (2). Tables 1 and 2 below list the most common cancers and cancer deaths by gender in the US in decreasing order of frequency.

Table 1: Most common cancers by gender (2)

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prostate</td>
<td>1. Breast</td>
</tr>
<tr>
<td>2. Lung &amp; bronchus</td>
<td>2. Lung &amp; bronchus</td>
</tr>
<tr>
<td>3. Colon &amp; rectum</td>
<td>3. Colon &amp; rectum</td>
</tr>
</tbody>
</table>

Source: American Cancer Society. Cancer facts and figures 2009
Table 2. Most common causes of cancer deaths by gender (2)

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lung &amp; bronchus</td>
<td>1. Lung &amp; bronchus</td>
</tr>
<tr>
<td>2. Prostate; Colon &amp; rectum</td>
<td>2. Breast</td>
</tr>
<tr>
<td>3. Pancreas</td>
<td>3. Colon &amp; rectum</td>
</tr>
</tbody>
</table>

Source: American Cancer Society. Cancer facts and figures 2009

With respect to race and ethnicity, African American males have a higher incidence of cancers in general compared to white males (2). In contrast, white females have a higher incidence of cancers compared to African American females (2). Table 3 shows the incidence rates in males and females in the USA by race and ethnicity from 2001-2005.

Table 3: Incidence of cancer by race and ethnicity (2)

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Americans</td>
<td></td>
</tr>
<tr>
<td>651.5 per 100,000</td>
<td>398.9 per 100,000</td>
</tr>
<tr>
<td>Whites</td>
<td></td>
</tr>
<tr>
<td>551.4 per 100,000</td>
<td>423.6 per 100,000</td>
</tr>
</tbody>
</table>

Source: American Cancer Society. Cancer facts and figures 2009

Thus, the high incidence rates of cancer and deaths due to cancer warrant further exploration into the disease processes associated with this highly morbid condition.
Cancer and cachexia

About 50-80% of patients diagnosed with cancer develop cachexia (2). The term cachexia is derived from the Greek words “kakos” meaning “bad” and “hexis” meaning “condition” (32). It is a multifactorial syndrome complex characterized by progressive weight loss and depletion of adipose tissue and skeletal muscle mass (14). In addition to cancer, cachexia may be seen in various chronic disease states—such as AIDS, malabsorption, cardiac failure, and renal failure—severe sepsis, major trauma, and surgery (32,30). As the associations and implications of cachexia are manifold, it is important to understand the pathophysiology of this syndrome.

Cachexia-Anorexia syndrome: Pathophysiology

Anorexia is defined as a loss or decrease in appetite (9,31). Many biochemical mediators (32,30,31,26,33) may trigger this condition and include:

1. Cytokines such as interleukin-1b, interleukin-6, interleukin-8, Interferon-gamma, Tumor necrosis factor- α (TNF-α), and ciliary neurotrophic factor.

2. Serotonin

3. Hypothalamic neuropeptides—such as neuropeptide Y and corticotropin releasing factor (CRF)

4. Peptide hormones—such as insulin, glucagon, and leptin (32,30,31,33).

Anorexia is often associated with cachexia (8) and may contribute to the latter.

The condition is referred to as the cachexia/anorexia syndrome. Figure 1 illustrates that although weight loss occurs in both starvation and cancer cachexia, the biochemical processes involved are not the same. It has been documented (32,30,31,33) that starvation
results predominantly in depletion of adipose tissue, in contrast to cachexia where there is increased catabolism and thereby depletion of both skeletal muscle and adipose tissue equally. Also, aggressive nutritional supplementation has not been successful in reversing cancer cachexia, implying that starvation (secondary to anorexia) is not solely responsible for the former (33).

Figure 1: Pathophysiology of cachexia (32,30,31,33,22)
As it is now clear that anorexia is only part of the spectrum of the cachexia syndrome, it is imperative to study the other probable factors contributing to the same.

**Testosterone and cancer cachexia**

Testosterone is the principal male sex hormone secreted predominantly by the Leydig cells of the testis (12). A small amount is produced by extraglandular conversion of androstenedione secreted by the testis and adrenal gland (19). Testosterone exerts its action either directly or after conversion into Dihydrotestosterone (DHT).

Effects of testosterone (12) are as follows:

1. Enhances libido, sexual desire, and fantasy.
2. Periosteal bone deposition resulting in cortical bone thickening and increase in bone mineral density and strength.
3. Maintains cancellous bone mass and expands cortical bone thus protecting against osteoporosis.
4. Increases muscle mass and strength
5. Improves mood- causes a sense of well-being.

Hypogonadism is defined as the condition in which production of sex hormones and germ cells (sperm and eggs) is inadequate (1). Low testosterone levels lead to decreased erectile function, decreased sexual desire, decreased cognition, tiredness, lack of motivation, sleep
disturbances, decreased spatial cognition, decreased quality of life, increased vasomotor flushes, decreased fat mass, and decreased bone and muscle mass (24). Patients with cancer cachexia develop similar symptoms such as skeletal muscle wasting, loss of fat mass, fatigue, depression, and sexual dysfunction (15). Chlebowski et al reported a 43% decrease in total testosterone and a 66% decrease in free testosterone in patients with metastatic cancer prior to chemotherapy (7). In a study by Strasser et al it was concluded that hypogonadism and symptoms of anorexia/cachexia are commonly seen in patients with advanced cancer (29). The results of a study (11) by our group with 31 male cancer patients and 25 gender matched controls showed a 90% prevalence of hypogonadism in male cancer patients (11). These studies raise an important question:

Is there a common mediator(s) in patients with low testosterone levels and in patients with cancer cachexia which result in the above symptoms?

The role of inflammatory mediators

The correlation between testosterone and inflammatory mediators in older men was studied by Marcello et al among others. A significant inverse relationship was noted between the levels of testosterone and an inflammatory mediator- soluble Interleukin- 6r (sIL-6r) (20). A randomized single-blind placebo controlled trial with a sample size of 25 patients, showed that administration of testosterone in androgen deficient males results in a significant decrease in the levels of TNF-alpha (21). Several other studies done in vitro and on animal models produced similar results (4,7). However, not many studies have been done studying the association between inflammatory markers and testosterone in cancer patients. The primary study has been undertaken to address this issue.
Public Health Significance

According to the National Institutes of Health estimates in 2008, a total of $228.1 billion was spent in the USA on cancer care (2). The average number of person years lost in cancer is approximately 15.5 (25). Despite recent advances in cancer treatment, there has not been a significant improvement in the quality of life of these patients (31). Determining the prevalence of hypogonadism in male cancer patients and the association of the symptoms of cancer cachexia and hypogonadism is of significance because evidence suggests that administration of testosterone in aging men increases muscle strength, improves fatigue, enhances mood, and improves quality of life (3,10,18,21,23,27,28). In addition, it has been documented that testosterone administration in AIDS patients with cachexia results in a significant gain in fat free mass, lean body mass, and muscle mass respectively (13). Thus, a high prevalence of hypogonadism in male cancer patients could prompt administration of testosterone in future randomized controlled trials to help alleviate symptoms of cachexia.

Research Questions and Hypotheses

Research Question 1: Is the proportion of patients with hypogonadism different in the male cancer and control groups?

Hypothesis 1: The proportion of patients with hypogonadism is significantly higher in the male cancer group when compared to the control group.

Research question 2: Are the levels of testosterone in male cancer patients with cachexia different from that of those without cachexia?

Hypothesis 2: The levels of testosterone in patients with cachexia are significantly lower
than that of patients without cachexia.

METHODS
The primary study, a confirmatory analysis, is titled “Prevalence of Hypogonadism in male cancer patients.” The Principal Investigator of the study is Jose M. Garcia M.D. The study began in November 2006 and is estimated to be completed by July 2010. It was approved by the Institutional Review Board of Baylor College of Medicine and is being conducted at the Michael E. DeBakey Veterans Affairs Medical Center at Houston. This paper is a pilot data analysis that employs a smaller subset convenience sample of 72 patients determined by using the data available for the 72 patients (of the intended sample of 135 patients) recruited between November 2006 and January 2010. As this is an exploratory analysis with a small sample size, the results should be interpreted with caution and may change when the analysis is done with the complete sample, once obtained.

Design of primary study: Cross-sectional

Sample size of primary study: 135

Sample size of the current exploratory data analysis: 72

Definition of terms:

1. **Cancer Cachexia (CC)**: Cancer patients who have lost 5% or more body weight in the past 6 months (30).

2. **Cancer non-cachexia (CNC)**: Cancer patients not included in group 1.

3. **Controls (Co)**: Patients who have never been diagnosed with any type of cancer.

4. **Sex-hormone binding globulin**: A carrier protein which binds to sex hormones
like Testosterone and makes them non-bioavailable (31).

5. **Free Testosterone (FT)**: The biologically active form of testosterone which is not bound to protein molecules such as sex-hormone binding globulin and albumin (31).

6. **Bioavailable Testosterone (BT)**: The fraction of testosterone that readily enters cells. It represents the total of free testosterone levels (FT) and the testosterone bound weakly to albumin (31). It can be assayed or calculated. If calculated, it is called **Calculated Bioavailable Testosterone (cBT)**.

7. **Total Testosterone (TT)**: The sum of free testosterone (FT) and protein-bound testosterone levels (31).

\[
TT = \text{Free Testosterone (FT)} + \text{Testosterone bound to Sex-Hormone Binding Globulin} + \text{Testosterone bound weakly to albumin}
\]

A previous report (17) showed that patients in the cancer group had a “Calculated Bioavailable Testosterone” (cBT) level of 42±25 ng/dl and 87±44 ng/dl in the control group (17). It was concluded that a sample size of 45 in each group, with a standard deviation of 44 ng/dl would be sufficient to detect a statistically significant difference with a power of 95% and an alpha value of 0.002 (17). However, as the dataset as of January 2010, had testosterone levels available for 72 patients, a sample size of 72 was chosen for the proposed analysis. This includes 50 patients in the cancer group and 22 gender, age, and BMI matched controls. The controls for the primary study were randomly selected from the out-patient male population of the Michael E. DeBakey VA Medical Center.
Inclusion criteria:

1. Male subjects aged 40-70 years with or without a histological diagnosis of cancer
2. Provision of written informed consent prior to screening.

Exclusion criteria:

1. BMI greater than 35 kg/m².
2. Evidence of ascites or clinically significant edema (that could confound assessment of body weight).
3. Active excessive alcohol intake or illicit drug abuse (because this has a known effect on the gonadal axis).
4. Concomitant Medications such as:
   a. Growth Hormone, Megestrol, Marinol, or any other anabolic agent, appetite stimulants (including corticosteroids other than dexamethasone at the time of IV chemotherapy administration), tube feedings, or parenteral nutrition during the 3 months prior to enrollment into the study.
   b. Participation in a clinical trial with investigational agents within 1 month of enrollment.
   c. Use of other medications that interfere with gonadal axis (androgens, estrogens, anti-androgens, etc).
   c. Patients diagnosed with non-melanomatous skin cancer (does not cause cachexia).

Procedures:

Once informed consent was obtained, the following procedures were done:

1. Blood Sample collection: An early morning, 8-hour fasting blood sample
was obtained to measure the levels of testosterone and sex hormone binding globulin, and albumin.

2. Body weight measurement: Measured in order to categorize cancer patients into cancer cachexia and cancer non-cachexia sub-groups.

Measurement of Total Testosterone:

The levels of total testosterone were measured using a radioimmunoassay kit (Diagnostic Products Cooperation, Los Angeles, CA) with $^{125}$I testosterone as the tracer (17). The lower limit of detection is 0.6914 ng/L using this method; with an intraassay coefficient of variation of 4.5%. Sex-Hormone Binding Globulin was measured using a 2-site immunoradiometric assay kit (IRMA; Diagnostic Systems Laboratories, Webster, TX) with $^{125}$I as the tracer. The lower limit of detection is 4 nmol/L with an intraassay coefficient of variation of 4.6% (17).

Measurement of Free Testosterone (FT) and Biologically active Testosterone (BT):

Free testosterone (FT) and Biologically active testosterone (BT) were calculated using the mass action equation as the resulting values correlate well with those obtained following reliable methods such as ammonium sulfate precipitation (17).

Sample Size Calculation and/or Study Power

If the prevalence of hypogonadism in male cancer patients is taken to be approximately 55% (6).

$$n = \frac{t^2pq}{d^2}$$
where \( n \) = required sample size

\[ t = \text{standard error} = 1.96 \]

\[ p = \text{prevalence of hypogonadism in male cancer patients} = 0.55 \]

\[ q = 1 - p = \text{prevalence of hypogonadism in non-cancer controls} = 1 - 0.55 \]

\( d \) = degrees of freedom

Thus, \( n = 1.96 \times 1.96 \times 0.55 (1-0.55) / 0.1 \times 0.1 \)

\[ = 95.0796 \]

If non-response is taken to be 10%,

Required sample size = \( 95.0796 / (1-0.1) \)

\[ = 105.644 \]

The sample size selected for the primary study was 135.

The sample size of the current analysis is 72 patients, with 50 patients in the cancer group and 22 patients in the control group.

Power of the current data analysis is calculated to be 0.1816.

**Data Analysis**

The dataset is derived from the primary study “Prevalence of Hypogonadism in male cancer patients” – Principal investigator: Jose M. Garcia, M.D. Data analysis has been done using the STATA/IC version 11 software for Windows on de-identified data of a sub-sample of 72 study subjects.

- Comparing the proportion of patients with hypogonadism in the cancer and control groups:
Table 4 shows the number of patients with hypogonadism (calculated bioavailable testosterone levels less than 70 ng/dl) in the cancer and non-cancer groups.

Exposure variable: Cancer
Outcome variable: Hypogonadism

The cut-off value for calculated bioavailable testosterone level is 70 ng/dl (16).

The rationale for using this cut off is that symptoms of hypogonadism occur below this level. In fact, most researchers have used this cutoff in studies on male hypogonadism (16). Patients with calculated bioavailable testosterone below 70 ng/dl are considered to be hypogonadal.

Table 4: STATA Output- Pearson’s chi2 test (cases= cancer patients; controls= non-cancer patients)

<table>
<thead>
<tr>
<th>Hypogonadism</th>
<th>No hypogonadism</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>16.7</td>
<td>33.3</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Pearson chi2(1) = 1.6036  *Pr = 0.205

Thus, the proportion of patients with hypogonadism in the male cancer group is
47.5% and the proportion of patients with hypogonadism in the non-cancer control group is 22.7%.

- Comparing the levels of calculated Bioavailable testosterone in male cancer patients and controls:

Testing for the normality of data:

Table 5: Shapiro-Wilk W test for normality of data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>W</th>
<th>V</th>
<th>z</th>
<th>Prob&gt;z</th>
</tr>
</thead>
<tbody>
<tr>
<td>cancer</td>
<td>50</td>
<td>0.96460</td>
<td>1.665</td>
<td>1.087</td>
<td>0.13854</td>
</tr>
<tr>
<td>control</td>
<td>22</td>
<td>0.90176</td>
<td>2.489</td>
<td>1.849</td>
<td>0.03224</td>
</tr>
</tbody>
</table>

From the above STATA output, we can conclude that the data of the control group is not normally distributed. Therefore, we logarithmically transform both cancer and control groups and re-check normality of the transformed data.

“lncontrol” is the logarithmic transformation of “control”

“lncancer” is the logarithmic transformation of “cancer”

Table 6 below shows the STATA output for testing normality of the transformed data. We can see that the p value is not significant, implying that the data is now normally distributed.

Table 6: Shapiro-Wilk W test for normality of transformed data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>W</th>
<th>V</th>
<th>z</th>
<th>Prob&gt;z</th>
</tr>
</thead>
<tbody>
<tr>
<td>lncancer</td>
<td>50</td>
<td>0.96577</td>
<td>1.610</td>
<td>1.016</td>
<td>0.15493</td>
</tr>
<tr>
<td>lncontrol</td>
<td>22</td>
<td>0.91387</td>
<td>2.182</td>
<td>1.582</td>
<td>0.05681</td>
</tr>
</tbody>
</table>
Table 7 shows the STATA output of the Variance ratio test of the two transformed groups. We see that the variances are unequal. Therefore we perform the t test for unequal variances as shown in Table 8.

Table 7: Variance ratio test of transformed data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>incancer</td>
<td>50</td>
<td>6.406388</td>
<td>.0119342</td>
<td>.0843877</td>
<td>6.382405 6.430371</td>
</tr>
<tr>
<td>lncontrol</td>
<td>22</td>
<td>4.557691</td>
<td>.0868695</td>
<td>.4074541</td>
<td>4.377036 4.738346</td>
</tr>
<tr>
<td>combined</td>
<td>72</td>
<td>5.841508</td>
<td>.1047109</td>
<td>.8885015</td>
<td>5.632721 6.050296</td>
</tr>
</tbody>
</table>

ratio = sd(incancer) / sd(lncontrol) f = 0.0429

Ho: ratio = 1 degrees of freedom = 49, 21

Ha: ratio < 1 Ha: ratio != 1 Ha: ratio > 1

Pr(F < f) = 0.0000 2*Pr(F < f) = 0.0000 Pr(F > f) = 1.0000

From the above STATA output, we can conclude that the variances are unequal. Therefore, we run the two-sample t test with unequal variances.

Table 8: Two-sample t test with unequal variances

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>incancer</td>
<td>50</td>
<td>6.406388</td>
<td>.0119342</td>
<td>.0843877</td>
<td>6.382405 6.430371</td>
</tr>
<tr>
<td>lncontrol</td>
<td>22</td>
<td>4.557691</td>
<td>.0868695</td>
<td>.4074541</td>
<td>4.377036 4.738346</td>
</tr>
<tr>
<td>combined</td>
<td>72</td>
<td>5.841508</td>
<td>.1047109</td>
<td>.8885015</td>
<td>5.632721 6.050296</td>
</tr>
<tr>
<td>dif</td>
<td>1</td>
<td>1.848697</td>
<td>.0876854</td>
<td>1.66675</td>
<td>2.030643</td>
</tr>
</tbody>
</table>

dif = mean(incancer) - mean(lncontrol) t = 21.0833
Ho: diff = 0                     Satterthwaite's degrees of freedom = 21.7968

Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0

Pr(T < t) = 1.0000         *Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000

- Analyzing sub-groups within the cancer group based on cachexia

The STATA output in Table 9 ascertains that the data is normally distributed.

Table 9: Shapiro-Wilk W test for testosterone, free testosterone (ft) and Bioavailable testosterone (bt) levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>W</th>
<th>V</th>
<th>z</th>
<th>Prob&gt;z</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone</td>
<td>50</td>
<td>0.98745</td>
<td>0.590</td>
<td>-1.125</td>
<td>*0.86967</td>
</tr>
<tr>
<td>ft</td>
<td>50</td>
<td>0.96610</td>
<td>1.594</td>
<td>0.995</td>
<td>*0.15995</td>
</tr>
<tr>
<td>bt</td>
<td>50</td>
<td>0.96460</td>
<td>1.665</td>
<td>1.087</td>
<td>*0.13854</td>
</tr>
</tbody>
</table>

As the data is normally distributed, a two-sided unpaired t-test for independent samples is done, prior to which, the variance ratio test is done as seen in tables 11-13 below.

Independent variable: Cachexia (categorical variable)

Dependent variable: Testosterone level (continuous variable)

Null Hypothesis (H₀): Testosterone levels are equal in the cancer cachexia (CC) and cancer non-cachexia (CNC) group.

Alternative Hypothesis (Hₐ): Testosterone levels in the cancer cachexia (CC) group are different when compared to that in the cancer non-cachexia (CNC) group.

Table 10 below, illustrates the summary statistics of cancer cachexia and cancer non-cachexia sub-groups.
Table 10: Summary statistics: mean, sd, N by categories of: group (Group)

0: cancer non-cachexia; 1: cancer cachexia

<table>
<thead>
<tr>
<th>Group</th>
<th>testos~e</th>
<th>Ft</th>
<th>bt</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.927188</td>
<td>5.094688</td>
<td>103.5094</td>
</tr>
<tr>
<td></td>
<td>1.69618</td>
<td>2.199164</td>
<td>48.5092</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>1</td>
<td>3.578333</td>
<td>4.168889</td>
<td>84.07778</td>
</tr>
<tr>
<td></td>
<td>1.706035</td>
<td>2.556445</td>
<td>55.0637</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>3.8016</td>
<td>4.7614</td>
<td>96.514</td>
</tr>
<tr>
<td></td>
<td>1.690727</td>
<td>2.351299</td>
<td>51.27783</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Tables 11-13 below, show the STATA output of the variance ratio test for Bioavailable testosterone, testosterone, free testosterone, and SHBG levels respectively. A p value >0.05 implies that the variances are equal for each of the groups.

Table 11: For Bioavailable testosterone, comparing variances by group (X= cancer non-cachexia, Y= cancer cachexia)

Variance ratio test

<table>
<thead>
<tr>
<th></th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>32</td>
<td>103.5094</td>
<td>8.575296</td>
<td>48.5092</td>
<td>86.01997 - 120.9988</td>
</tr>
<tr>
<td>Y</td>
<td>18</td>
<td>84.07778</td>
<td>12.97864</td>
<td>55.0637</td>
<td>56.69525 - 111.4603</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>96.51402</td>
<td>7.251781</td>
<td>51.27784</td>
<td>81.94102 - 111.087</td>
</tr>
</tbody>
</table>

\[ \text{ratio} = \frac{\text{sd}(x)}{\text{sd}(y)} \quad f = 0.7761 \]

\[ \text{Ho: ratio} = 1 \quad \text{degrees of freedom} = 31, 17 \]

\[ \text{Ha: ratio} < 1 \quad \text{Ha: ratio} != 1 \quad \text{Ha: ratio} > 1 \]

\[ \Pr(F < f) = 0.2625 \quad 2*\Pr(F < f) = 0.5249 \quad \Pr(F > f) = 0.7375 \]
Table 12: For free testosterone, comparing the variances by group

Variance ratio test

<table>
<thead>
<tr>
<th></th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>32</td>
<td>5.094688</td>
<td>.3887609</td>
<td>2.199164</td>
<td>4.301805 5.887571</td>
</tr>
<tr>
<td>Y</td>
<td>18</td>
<td>4.168889</td>
<td>.6025599</td>
<td>2.556445</td>
<td>2.897599 5.440179</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>4.7614</td>
<td>.3325239</td>
<td>2.351299</td>
<td>4.093168 5.429632</td>
</tr>
</tbody>
</table>

ratio = sd(x) / sd(y)  

f = 0.7400

Ho: ratio = 1

Ha: ratio < 1                         Ha: ratio != 1                       Ha: ratio > 1

Pr(F < f) = 0.2270                2*Pr(F < f) = 0.4541           Pr(F > f) = 0.7730

Table 13: For total testosterone, comparing the variances by group

Variance ratio test

<table>
<thead>
<tr>
<th></th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>32</td>
<td>3.927188</td>
<td>.2998451</td>
<td>1.69618</td>
<td>3.31565  4.538726</td>
</tr>
<tr>
<td>Y</td>
<td>18</td>
<td>3.578333</td>
<td>.4021163</td>
<td>1.706035</td>
<td>2.729942 4.426724</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>3.8016</td>
<td>.2391048</td>
<td>1.690726</td>
<td>3.321101 4.282099</td>
</tr>
</tbody>
</table>

ratio = sd(x) / sd(y)  

f = 0.9885

Ho: ratio = 1

Ha: ratio < 1                      Ha: ratio != 1                      Ha: ratio > 1

Pr(F < f) = 0.4726          2*Pr(F < f) = 0.9453           Pr(F > f) = 0.5274

As the variances are equal, we now perform the two sample t test with equal variances, as shown in Tables 14-16.
Table 14: Two-sample t test with equal variances (Bioavailable testosterone)

<table>
<thead>
<tr>
<th>Group</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>103.5094</td>
<td>8.575295</td>
<td>48.5092</td>
<td>86.01994 120.9988</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>84.07778</td>
<td>12.97864</td>
<td>55.0637</td>
<td>56.69524 111.4603</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>96.514</td>
<td>7.251781</td>
<td>51.27783</td>
<td>81.941 111.087</td>
</tr>
<tr>
<td>Diff</td>
<td></td>
<td>19.4316</td>
<td></td>
<td>15.00456</td>
<td>-10.73709 49.60028</td>
</tr>
</tbody>
</table>

diff = mean(0) - mean(1)  
\[ t = 1.2950 \]

Ho: diff = 0  
degrees of freedom = 48

Ha: diff < 0  
Ha: diff != 0  
Ha: diff > 0

Pr(T < t) = 0.8993  
*Pr(|T| > |t|) = 0.2015  
Pr(T > t) = 0.1007

Table 15: Two-sample t test with equal variances (Free testosterone)

<table>
<thead>
<tr>
<th>Group</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>5.094688</td>
<td>.3887609</td>
<td>2.199164</td>
<td>4.301805 5.887571</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>4.168889</td>
<td>.6025599</td>
<td>2.556445</td>
<td>2.897599 5.440179</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>4.7614</td>
<td>.3325239</td>
<td>2.351299</td>
<td>4.093168 5.429632</td>
</tr>
<tr>
<td>Diff</td>
<td></td>
<td>.925799</td>
<td>.6870632</td>
<td>-</td>
<td>-.4556341 2.307232</td>
</tr>
</tbody>
</table>

diff = mean(0) - mean(1)  
\[ t = 1.3475 \]

Ho: diff = 0  
degrees of freedom = 48

Ha: diff < 0  
Ha: diff != 0  
Ha: diff > 0

Pr(T < t) = 0.9079  
*Pr(|T| > |t|) = 0.1842  
Pr(T > t) = 0.0921
Table 16: Two-sample t test with equal variances (Total testosterone)

<table>
<thead>
<tr>
<th>Group</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>3.927188</td>
<td>.2998451</td>
<td>1.69618</td>
<td>3.31565 – 4.538726</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>3.578333</td>
<td>.4021163</td>
<td>1.706035</td>
<td>2.729942 – 4.426724</td>
</tr>
<tr>
<td>Combined</td>
<td>50</td>
<td>3.8016</td>
<td>.2391048</td>
<td>1.690726</td>
<td>3.321101 – 4.282099</td>
</tr>
<tr>
<td>Diff</td>
<td></td>
<td>.348855</td>
<td>.5007721</td>
<td>.6580148</td>
<td>1.355725</td>
</tr>
</tbody>
</table>

\[ \text{diff = mean(0) - mean(1)} \quad t = 0.6966 \]

Ho: diff = 0  
Ha: diff < 0  
Ha: diff ! = 0  
Ha: diff > 0  

\[ \Pr(T < t) = 0.7553 \quad *\Pr(|T| > |t|) = 0.4894 \quad \Pr(T > t) = 0.2447 \]

**Human Subjects, Safety Considerations**

This research study is a pilot data analysis that employs a smaller subset convenience sample of 72 patients determined by using the data available for the 72 patients of the intended sample of 135 patients of the primary study “Prevalence of hypogonadism in male cancer patients” – recruited between November 2006 and January 2010. The study was approved by the Institutional Review Board of Baylor College of Medicine and was exempt from review by the IRB of the University Of Texas- School Of Public Health. All data provided to me was de-identified and had no personal identifiers.

**RESULTS**

Table 17 below gives the summary of results in the cancer and control groups.
Table 17: Summary of results: Cancer versus control group:

<table>
<thead>
<tr>
<th></th>
<th>Cancer group (n=50)</th>
<th>Control group (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of proportion of patients with hypogonadism</td>
<td>47.5%</td>
<td>22.7%</td>
</tr>
<tr>
<td>Pearson chi2(1) =</td>
<td>1.6036</td>
<td>Pr = 0.205</td>
</tr>
<tr>
<td>Comparison of cBT levels</td>
<td>94.56</td>
<td>108.4</td>
</tr>
<tr>
<td>t statistic =21.08, p value&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 18 below summarizes the results in the cancer cachexia and non-cachexia groups.

Table 18: Summary of results: Cancer Cachexia versus Cancer non-cachexia groups:

<table>
<thead>
<tr>
<th></th>
<th>Cancer cachexia (n=18)</th>
<th>Cancer non-cachexia (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean total testosterone</td>
<td>3.58</td>
<td>3.93</td>
</tr>
<tr>
<td>t statistic = 0.6966, p value = 0.4894</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean free testosterone</td>
<td>4.17</td>
<td>5.09</td>
</tr>
<tr>
<td>t statistic = 1.3475, p value = 0.1842</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean bioavail testosterone</td>
<td>84.08</td>
<td>103.51</td>
</tr>
<tr>
<td>t statistic = 1.2950, p value = 0.2015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The above data analysis is only a preliminary one and the results are subject to change once the complete dataset is available. As the data in the control group was not normally distributed, logarithmic transformation was employed to the data of both cancer and control groups, ascertaining normality afterward. The proportion of patients with hypogonadism in the cancer group was not different from that in the control group. Inferences on prevalence of hypogonadism in male cancer patients cannot be made in view of the small sample size and thereby lack of generalizability of this subset of patients. However, the mean level of calculated bioavailable testosterone in male cancer patients was lower than that of non-cancer controls and the difference reached statistical significance. Within the cancer group,
cachectic and non-cachectic patients were analyzed with respect to calculated Bioavailable testosterone, free testosterone, and total testosterone levels. In contrast to earlier studies (8,11,29) the current analysis did not reveal any statistical significant difference in testosterone levels (calculated Bioavailable testosterone, free testosterone, and total testosterone) between the cancer cachexia and cancer non-cachexia groups. Despite well-defined inclusion and exclusion criteria set at the beginning of the study, and objective measures of analysis, the results of this exploratory study require cautious interpretation. The small sample size resulting in a low power is the major limiting factor of the current analysis. A sample size of 106 would have achieved adequate power. Also, as the number of subjects in the cancer cachexia and non-cachexia sub-groups was small, it was not feasible to control for cancer type and stage, which are important variables that may have confounded the results. Another important limiting factor of the study is the unequal distribution of patients in the cancer and control groups and in the cachexia and non-cachexia sub-groups. A 1:1 ratio of the cancer and control groups would have been ideal. Despite the above limitations, the current analysis serves as a good template to guide further research and analysis. The results of the primary study will help confirm or refute the inferences of this paper.

CONCLUSION

The exploratory nature of this study prevents us from drawing definitive conclusions. The results of this secondary data analysis did not completely concur with the proposed hypotheses. Though there was a significant difference in calculated bioavailable testosterone levels between male cancer patients and controls, the current analysis did not reveal a
statistical significant difference between the proportion of patients with hypogonadism in the cancer and non-cancer control groups. Also, there was no statistically significant difference in the testosterone levels (calculated Bioavailable testosterone, free testosterone, and total testosterone) between cancer cachexia and non-cachexia sub-groups within the cancer group. Despite the limitations of this analysis, the results raise an important question. If the difference in testosterone levels does not contribute to cachexia in male cancer patients, is it the inflammatory cytokines alone that contribute to the same? However, as stated above, the results of the current analysis are subject to change once the complete dataset is available. However, future large-scale randomized prospective studies are imperative for a better understanding of the association between cancer and hypogonadism.
REFERENCES


